Exhibit E

DOCKET NO. 2200.007
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
BRIGID L. M. HOGAN)
) Group Art Unit: 1802
Serial No. 07/958,562)
) Examiner: D. Saunders
Filed: October 8, 1992)
)
For: "PLURIPOTENTIAL EMBRYONIC)
STEM CELLS AND METHODS OF)
MAKING SAME")

DECLARATION OF BRIGID L. M. HOGAN PURSUANT TO 37 C.F.R. §1.132

Honorable Commissioner of
Patents and Trademarks

Washington, D.C. 20231

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January 17, 1995

I, Brigid L. M. Hogan, a citizen of the United Kingdom, residing at 103 Robert E. Lee Lane, Brentwood, Tennessee 37207, U.S.A., declare that:

1. I am the inventor of the above-captioned application identified as Serial Number 07/958,562.

2. As a follow-up experiment to the mouse experiment described in the above-captioned application my laboratory applied the procedures used for mice embryos to human embryos.

The above methods for isolation of ES cells from murine embryos were repeated for isolation primordial germ cells from

human embryos. Specifically, testes were dissected from a 10.5 week human embryo. Tissue was rinsed in buffered saline, and incubated in trypsin solution (0.25% trypsin, 1 mM EDTA in Ca^{++}/Mg^{++} free HEPES buffered saline) for 10 minutes at 37°C. The tissue was dissociated by pipetting and the cells plated into wells of a 24 well tray containing irradiated feeder cells. In this experiment the feeder cells were Sl/Sl mouse fibroblasts transfected with human membrane associated Stem Cell Factor (Sl^{h}h220 cells from Dr. David Williams, HHMI, Indiana State University School of Medicine). The culture medium consists of Dulbecco's modified Eagle's medium (DMEM) with 15% fetal bovine serum supplemented with 10 ng/ml human bFGF, 60 ng/ml human Stem Cell Factor and 10 ng/ml human LIF. The cultures were maintained for 5 days, with daily addition of fresh growth factors.

After 5 days, cultures were dissociated with trypsin solution as before and seeded into wells containing a feeder layer of irradiated Sl^{h}h220 embryo fibroblasts. The medium was supplemented with growth factors daily as above.

After 10 days the cultures were fixed and stained for alkaline phosphatase activity. Colonies of cells expressing high levels of alkaline phosphatase and closely resembling primordial germ cells of the mouse embryo were detected in many wells (see Figure 5). Closely packed clusters of cells were present in some colonies (arrow in Figure 5). In cultures of mouse embryo germ cells these colonies give rise to lines of pluripotential embryonic stem cells.

3. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and, further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United

States Code and that such wilful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

Jan 16 1995 — Brigid Hogan

Date — Brigid M. M. Hogan

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